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Poly(oligo (ethylene glycol) vinyl acetate)s: A versatile new class of thermoresponsive and biocompatible polymers

Guillaume G. Hedir,[a][b] Maria C. Arno,[a] Marvin Langlais,[a] Jonathan T. Husband,[a] Rachel K. O'Reilly*[a] and Andrew P. Dove*[a]

Abstract: Polymers with a thermally-triggered phase transition are important in the design of materials for biological applications where their behavior can be used to trigger release or (dis)assembly events. Despite their advantages, a system with tuneable thermal response, end-group reactive sites, low toxicity and controlled main-chain degradability has not been realised, yet would represent a significant advance. We report the versatile new poly(oligo(ethylene glycol) vinyl acetate)s with excellent control over their molecular properties obtained through RAFT/MADIX polymerization. Furthermore, we demonstrate structure-controlled thermal transitions, conjugation to human lysozyme through the retained end group and moreover show that this class of polymers can uniquely be copolymerized with 2-methylene-1,3-dioxepane (MDO) to generate polymers in which the degradability and cloud point can be independently tuned to create materials that display the same cloud point but degrade differently.

In the last decade "smart" polymers that respond to external stimuli have widely been investigated as a consequence of their great potential in nanotechnology and biomedical applications.[1] The use of water-soluble polymers exhibiting a lower critical solution temperature (LCST)[2] in aqueous media provide a promising tool in the biomedical field where they can be used as smart bioactive surfaces, drug delivery systems or as tissue engineering scaffolds where transitions are commonly used to release small molecules.[1c, 3] Perhaps the most commonly studied thermoresponsive polymer that displays an LCST is poly(*N*-isopropylacrylamide), poly(NIPAm). While it has attracted significant attention as a consequence of the close proximity of its LCST to body temperature (32 °C), the toxicity of the *N*-isopropylacrylamide monomer, and subsequent polymer,[4] has highlighted the need to find new non-toxic alternatives.

Aoshima[5] and Lutz [6] with their respsctive coworkers developed a range of biocompatible and thermoresponsive polymers based on polymerization of di(ethylene glycol) vinyl ether- and acrylate-based monomers respectively. While these polymers have been widely investigated,[6b, 7] the degradation of the latter by hydrolysis to poly((meth)acrylic acid) presents potential issues with acidolysis in a biological environment. While other approaches to oligo(ethylene glycol) grafted polymers could be appealing,[8] one of the most promising alternatives are copolymers of vinyl alcohol (VA) and vinyl acetate (VAc), whose thermoresponsive properties depend on the degree of acetylation after controlled hydrolysis.[9] PVA-based polymers are often considered as ideal candidate materials in biomedical applications as a consequence of their high biocompatibility which has led to their FDA approval in a range of biomedical applications.[9c] However, while promising, poly(VAc-*co*-VA)-based polymers prepared using controlled radical polymerization methods lose end-group fidelity during the hydrolysis of the acetate groups, thus preventing their use in areas such as polymer-protein conjugation amongst others. Furthermore, their thermoresponsive properties are highly dependent on the degree of hydrolysis, where blocky polymer structures can result and in turn lead to undefined phase transitions and unpredictable thermal response.[10]

Clearly there is a need for an advanced thermally-responsive polymer with a defined tuneable transition and limited cytotoxicity for both the material and its potential degradation by-products to offer opportunities to create new materials for advanced biomedical applications. Herein, we report a new family of poly(VAc)-derived polymers that display a tuneable thermal response through both monomer structure and comonomer incorporation. When polymerized using reversible addition-chain transfer (RAFT) polymerization, good control over the molecular parameters can be obtained alongside high end group retention which enables their conjugation to proteins. Additionally, unlike more-active monomers (MAMs) such as (meth)acrylates or acrylamides, being a less-active monomer (LAM), enables random copolymerization with 2-methylene-1,3-dioxepane (MDO), a cyclic ketene acetal (CKA) that results in main chain esters through radical ring-opening polymerization (rROP) which results in the ability to create polymers with independently tuneable thermal response and degradative behaviour.

The new oligo (ethylene glycol) vinyl acetate monomer (MeO_2VAc, **2**) was obtained by reaction between the commercially available 2-[2-(2-methoxyethoxy)ethoxy] acetic acid and vinyl acetate using a palladium vinyl exchange reaction at 60 °C for 16 h (Scheme 1, Figures S1-S2). Such palladium vinyl exchange reaction represents a significant approach towards the production of functional vinyl ester-based materials.[11] Polymerization of MeO_2VAc, using RAFT/MADIX (macromolecular design by interchange of xanthates) polymerization, was performed in bulk at 90 °C for

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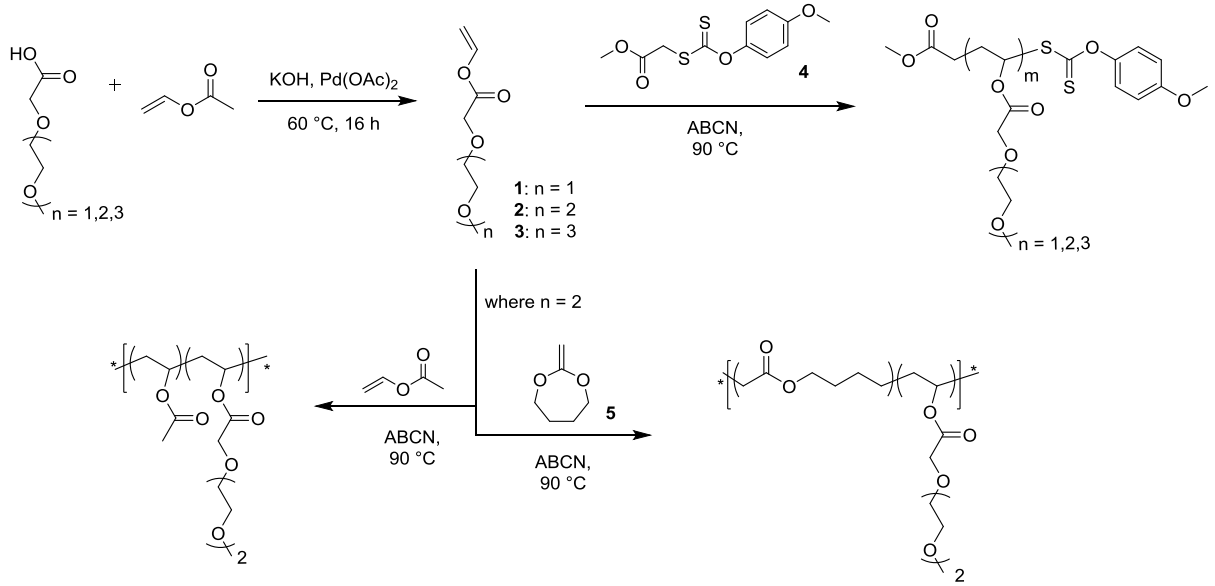

Scheme 1. Synthesis and (co)polymerization of MeO_nVAc using RAFT/MADIX polymerization.

different reaction times using 1,1'-azobis-(cyclohexanocarbonitrile) (ABCN) and *O*-*p*-methoxyphenyl xanthate, **4**,[12] as the chain transfer agent (CTA) (Scheme 1). SEC analysis of the resultant polymers revealed the successful formation of poly(MeO_2VAc) with dispersities ($Đ_M$) between 1.13 and 1.53 (Figures S3-S4, Table S1) and ^{1}H NMR spectroscopy affirmed good retention of the xanthate chain-end at δ = 7.00 – 6.90 ppm (Figure S5). Evaluation of the solubility and thermoresponsive properties of poly(MeO_2VAc) by dynamic and static light scattering (DLS/SLS) as well as turbidimetry confirmed the dissolution of poly(MeO_2VAc) in aqueous medium as observed by the unimeric population (Figures S6-S7), and the presence of a cloud point at 83 °C (Figure 1b). Investigation of the thermal transition of poly(MeO_2VAc) upon cooling and heating revealed no significant hysteresis effect (Figure S8) while the degree of polymerization (DP) was found to not affect the cloud point (Table S2, Figure S9). Comparatively, a change in polymer concentration (Figure S10) demonstrated an increase in cloud point as concentration was decreased below 5 mg/mL.

To extend the range of thermal transitions, ethylene glycol methyl ether vinyl acetate (MeOVAc) and tri(ethylene glycol) methyl ether vinyl acetate (MeO_3VAc) were isolated by comparable syntheses (Figures S11-S14). RAFT/MADIX polymerization of these monomers using similar reaction conditions produced poly(MeOVAc) and poly(MeO_3VAc) respectively (Figures S15-S18). As anticipated from analogy to the OEGMA polymer series, turbidimetry experiments on poly(MeOVAc) and poly(MeO_3VAc) revealed cloud points at 57 °C and 93 °C respectively (Figure 1a & 1c), therefore making this new class of thermoresponsive polymers applicable to a wide array of applications.

In order to demonstrate the high chain-end retention of this process, ^{1}H NMR and ^{13}C NMR spectra of poly(MeO_2VAc) were analyzed. These spectra confirmed the presence of xanthate end group through the characteristic signals at δ =

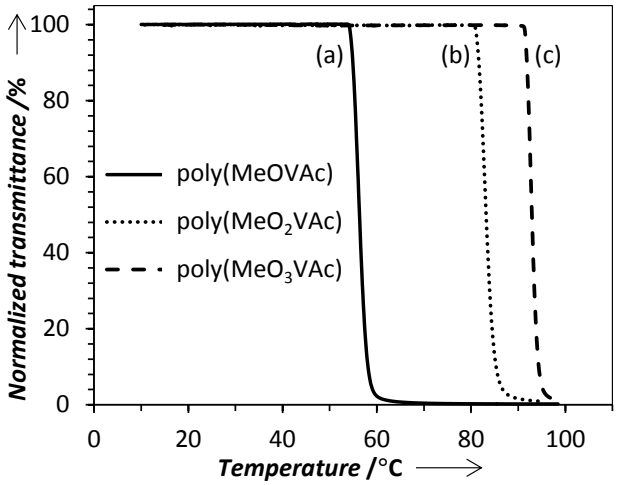

Figure 1. Intensity of the normalized transmitted laser light *vs*. temperature for (a) poly(MeOVAc), (b) poly(MeO_2VAc) and (c) poly(MeO_3VAc) at a concentration of 5 mg/mL in water.

7.00 – 6.90 ppm (Figure S19) and δ = 158.1, 147.1, 122.7, 114.6 and 55.6 (Figure S20) respectively. Furthermore, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) analysis observed a main distribution that corresponds to the sodium-charged, xanthate-terminated poly(MeO_2VAc) with spacing between peaks equal to the molar mass of the monomer repeat unit (*m/z* 204, Figure S21).

To further demonstrate the importance of this observation, we investigated the potential of poly(MeO_2VAc) for polymer/protein bioconjugation. Post-polymerization aminolysis of poly(MeO_2VAc) was conducted to convert the aromatic xanthate end-group to a thiol (Figures S22-S23) and found to have little effect on the thermal properties or the quality of the polymer sample (Figures S24-S25). Succinimidyl iodoacetate was utilized to form iodoacyl modified human lysozyme which was subsequently conjugated to the thiol functionalised polymer (Scheme S1). This resulted in a

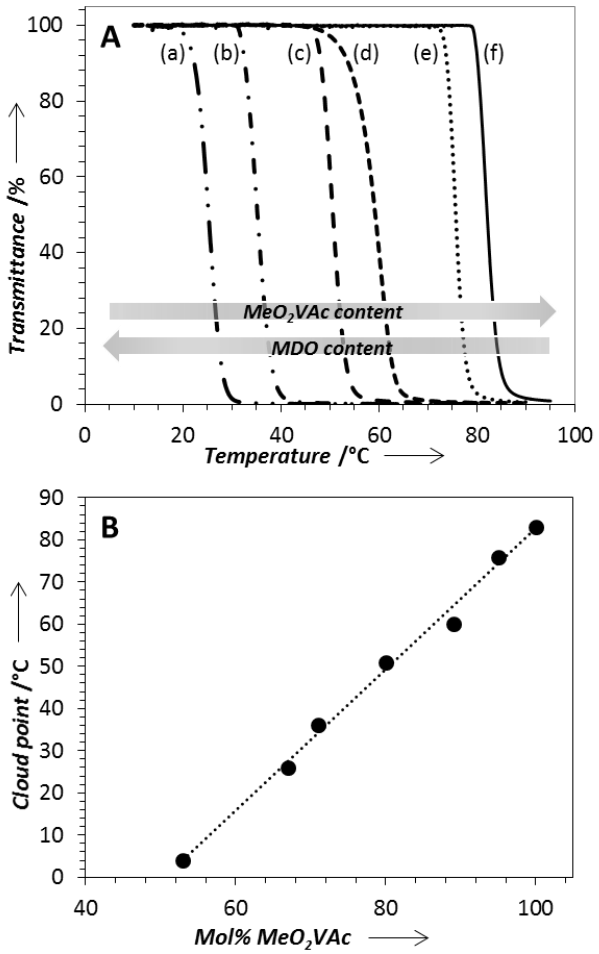

Figure 2. (A) Intensity of the normalized transmitted laser light *vs* temperature for the poly(MeO_2VAc-*co*-MDO) for varying compositions (a) 67 mol%, (b) 71 mol%, (c) 80 mol%, (d) 89 mol%, (e) 95 mol% and (f) 100 mol% of MeO_2VAc, at a concentration of 5 mg/mL in water and (B) influence of MeO_2VAc content on cloud point observed.

conjugate in excess of 100 kDa with minimal unreacted enzyme as observed by SDS-PAGE (Figure S26) which shows that this system is promising for protein-polymer conjugation applications with tuneable on/off activity.

In order to further extend and control the cloud point of this class of polymers, MeO_2VAc was copolymerized with other LAMs. Initial studies focussed on the copolymerization of MeO_2VAc with VAc to create a library of poly(MeO_2VAc-*co*-VAc) with increasing MeO_2VAc content (Table S3). While the presence of VAc in the polymerization mixture did not affect the nature of the polymerization (monomodal traces and high chain-end fidelity, Figures S27-S28), the incorporation of VAc within the copolymer was found to significantly influence the thermoresponsive properties. Indeed, while poly(MeO_2VAc) displayed a cloud point at 83 °C, the poly(MeO_2VAc-*co*-VAc)s had similar cloud points upon cooling and heating between 79 and 18 °C with those with lower MeO_2VAc content displaying greater depression in cloud point as a consequence of the increased hydrophobicity from the VAc segments (Figures S29-31).

While the development of MeO_nVAc monomers to create a family of new thermally-responsive copolymers with high end-

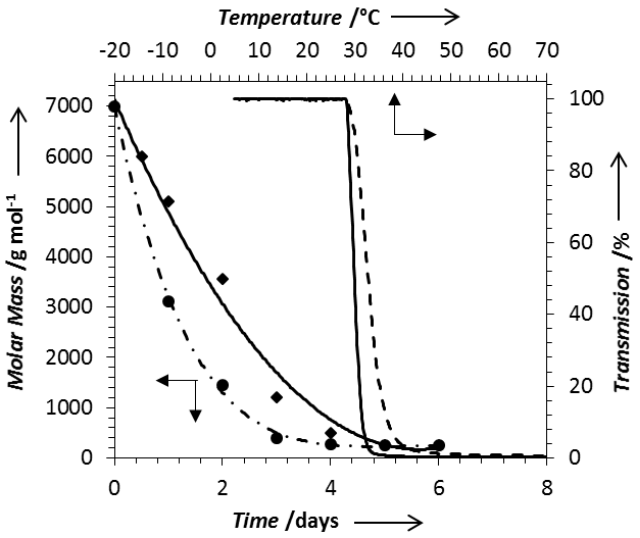

Figure 3. Intensity of the normalized transmitted laser light *vs* temperature (lines with no points) and residual molecular weight observed *vs* hydrolysis days (points and lines) for poly(MeO_2VAc-*co*-MDO) and poly(MeOVAc-*co*-MDO) with 33 and 10 mol% MDO (solid lines and dot/dash lines) respectively.

group fidelity represents a significant advance in the field, the use of a LAM presented a unique opportunity to incorporate degradability in a random manner by copolymerization of MeO_nVAcs with MDO, **5** which enables the formation of degradable polymers with a comparable structure to poly(ε-caprolactone),[13] that is widely accepted for biomedical applications unlike its more activated 5,6-benzo-2-methylene-1,3-dioxepane (BMDO) and 2-methylene-4-phenyl-1,3-dioxalane (MPDL) analogues.[14] Reactivity ratios near to 1 observed for copolymerization of MDO and VAc enables random placement of the degradable ester unit throughout the backbone,[13c, 15] unlike approaches that use MAMs such as acrylic monomers.[16] A series of poly(MDO-*co*-MeO_2VAc) samples were synthesized by RAFT/MADIX polymerization at 90 °C, (Table S4). SEC again observed monomodal peaks (Figure S32) and the xanthate chain-end functionality was retained (Figure S33) which indicates a well-controlled polymerization has occurred. Turbidimetry experiments on poly(MDO-*co*-MeO_2VAc)s revealed the presence of cloud points between 76 and 4 °C in a predictable manner based on the composition of the copolymers (Figure 2) while again no hysteresis was observed (Figure S34).

The different MeO_nVAc monomers present a unique opportunity to create a family of materials in which the cloud point can be tuned independently of the degradability. In order to demonstrate this principle, we synthesized two materials, poly(MDO-*co*-MeOVAc) and poly(MDO-*co*-MeO_2VAc) containing 10 and 33 mol% MDO respectively (Table S5), that were predicted to display similar cloud points (≈ 32 °C). The resultant polymers displayed cloud points of 29.9 and 32.9 °C respectively (Figure 3) as a result of their different MDO incorporation. Furthermore, comparison of initial monomer feed and final copolymer compositions suggested no significant differences in reactivity ratios between MDO and MeOVAc, MeO_2VAc and MeO_3VAc having different oligo

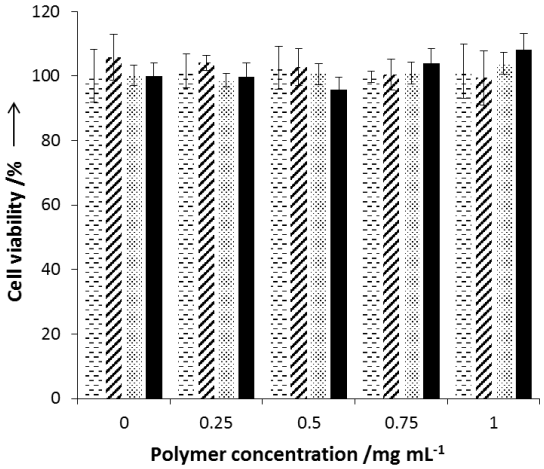

Figure 4. Viability of MC3T3 cells when incubated with poly(MeO_2VAc) (dashed horizontal fill), poly(MDO-*co*-MeO_2VAc) (diagonal fill) and the products of their degradation (dots and solid respectively) after 72 h, with a concentration range from 0 to 1 mg/mL.

(ethylene glycol) functionalities (Table S6). Upon exposure to enzymatic conditions (PBS solution, pH = 7.4, 37 °C, *lipase* immobilized from *Candida Antarctica* at 200U/g), degradation of the poly(MDO-*co*-MeO_2VAc) (33 mol% MDO) was significantly faster than for poly(MDO-*co*-MeOVAc) (10 mol% MDO) such that initial rates of degradation were double and time taken to degrade to 50% mass were halved for the polymer with lower MDO content (Figure 3). Such ability to design materials with independently tuneable thermal properties and degradation profile provide exciting possibilities for the advancement of degradable biomaterials.

Finally, in order to confirm that these materials and, importantly, their degradation products were non-cytotoxic, cell viability tests on MC3T3 (murine pre-osteoblasts) cells were undertaken. After 72 h, cell viabilities for all systems tested were not affected, being higher than 95% in the concentration range considered. This suggests that both poly(MDO-*co*-MeO_2VAc) and poly(MeO_2VAc) and their degradation products (Figure 4) are highly biocompatible and degrade to non-toxic byproducts, therefore re-enforcing their excellent candidature as degradable, thermally-responsive biomaterials.

This novel family of thermoresponsive polymers based on a PVAc scaffold present a new alternative to the well-known poly(NIPAm), poly(OEGMA) and poly(VAc-*co*-VA)-based polymers, which have all widely been considered as biomaterials in the biomedical field. The high biocompatibility, tuneable cloud point based on both monomer design and comonomer incorporation and high retention of end group from the RAFT/MADIX process to enable bioconjugation, all endow the family of poly(MeO_nVAc) with a significant advantage over any other analogous material. Moreover, the ability to create copolymers with degradable backbones by copolymerization with MDO highlights the unique opportunities defined by this system to independently control the thermal properties and degradation behaviour based on monomer choice and polymer composition.

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COMMUNICATION

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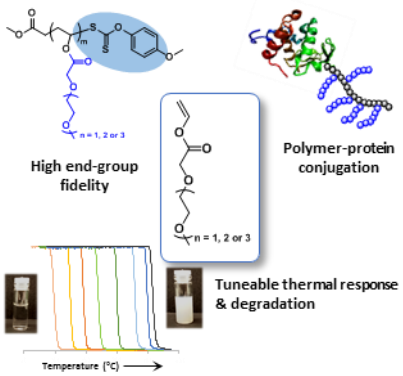

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